(12) UK Patent Application (19) GB (11) 2 218 989 (13) A

(43) Date of A publication 29.11.1989

(21) Application No 8829427.7

(22) Date of filing 15.12.1988

(30) Priority data
(31) 8729232 (32) 15.12.1987 (33) GB

(51) INT CL⁴
C07C 175/00, C07D 311/72, C07J 9/00

(52) UK CL (Edition J)
C2V V1 V23 V9

(56) Documents cited
GB 2148897 A GB 2090836 A GB 1562794 A

(58) Field of search
UK CL (Edition J) C2V, C5C CDD

(71) Applicants
Institut Penyelidikan Minyak Kelapa Sawit Malaysia

(Incorporated in Malaysia)

No 6 Pesiaran Institusi, Bandar Baru Bangi,
43000 Kajang, Selangor, Malaysia

University of Malaya
Lembah Pantai, 59100 Kuala Lumpur, Malaysia

(72) Inventors
Prof Swee Hock Goh
Dr Toh Seok Kam
Dr Yen May Choo
Prof Augustine Soon Hock Ong

(74) Agent and/or Address for Service
Boult Wade & Tennant
27 Furnival Street, London, EC4A 1PQ,
United Kingdom

(54) Recovery of carotenoids, tocopherols, tocotrienols and sterols from esterified palm oil

(57) The invention is for a method for the isolation of the minor non-glyceride components of palm oil or like vegetable oil containing free fatty acid and non-glyceride components similar to that of palm oil which method comprises:

(i) esterifying the free fatty acid component of the oil with one or more monohydric alcohols to form an esterified oil with a very low free fatty acid content,

(ii) converting the glycerides into monoesters by transesterification employing one or more monohydric alcohols,

(iii) adsorbing the non-glyceride components onto a selective absorbent to separate said components from the esters of the oil, and

(iv) thereafter desorbing the non-glyceride components from the adsorbent with the use of solvent to recover said components. The adsorbent is preferably activated alumina, activated carbon, or silica gel, preferably reverse phase (particularly C18) silica gel. By the method, carotenes, sterols, tocopherols and other non-glyceride components can be isolated.

GB 2 218 989 A

2218989

RECOVERY OF CAROTENOIDS, TOCOPHEROLS, TOCOTRIENOLS AND STEROLS FROM ESTERIFIED PALM OIL

Crude palm oil contains about 1% of non-glyceride components which include carotenoids, tocopherols, tocotrienols and sterols. The carotenoids, consisting of mainly α and β carotenes at 500 to 700 ppm, are important constituents with pro-vitamin A activity, possible anti-tumor formation properties, and other physiological activities. The tocopherols and tocotrienols are Vitamin E constituents and also natural anti-oxidants, and are present at approximately 600 to 1000 ppm in crude palm oil; the major component is the gamma-tocotrienol which has recently been found to have anti-cancer properties besides its known anti-oxidant activity. Tocotrienol has been found to lower blood cholesterol. (The sterols consists mainly of sitosterols, stigmasterol and campesterol provide raw materials for steroid intermediates and drugs).

Several methods have been developed to extract these valuable compounds. In the case of the carotenoids, the known methods can be classified as follows:-

(i) Extraction by saponification e.g. British Patent 567,682; U.S. Patent 2,460,796; U.S. Patent 2,440,029; U.S. Patent 2,572,467; U.S. Patent 2,652,433

(ii) Iodine method

(iii) Urea process

(iv) Extraction using Fuller's earth or activated carbon, e.g. British Patent 691,924; British Patent 1,563,794; U.S. Patent 2,484,040

(v) Extraction by selective solvents e.g. U.S. Patent 2,432,021

(vi) Molecular Distillation.

In the saponification method (i), the oil is saponified to give soap, glycerol and a non-saponifiable fraction containing carotenes.

In the iodine method (ii), the iodine is added to a solution of palm oil in petroleum ether, an insoluble precipitate of carotene di-iodide is formed. The iodo- compound when treated with sodium thiosulphate however yields iso-carotene or dehydro-caroten which has no biological activity.

With the urea method (iii), the triglycerides are broken down to fatty acids and methyl esters which then form insoluble compounds with urea and thiourea, leaving the carotenoids in the remaining liquid.

Extraction of carotenes using adsorbents has been carried out using Fuller's earth and activated carbon (method iv). However, the extraction of the carotenes from the earth gives oxidised or isomerised products of carotenes. Carotene is concentrated six times in the extract.

Extraction of carotenes by selective solvents (method v) has been carried out using propane or furfural. The carotene is concentrated (three times that of the original oil) in the furfural phase.

By method (vi) carotenes can also be obtained by molecular distillation (10^{-3} - 10^{-4} mm Hg). Fractions collected at 230^{0}C have a carotene content of about five times that of the original oil.

None of these methods however have been commercialised because of several difficulties.

According to the present invention there is a method for the isolation of the minor non-glyceride components of palm oil or like vegetable oil containing free fatty acid and non-glyceride components similar to that of plam oil, which method

comprises:

(i) Esterifying the free fatty acid component of the oil with one or more monohydric alchols to form an esterified oil with a very low free fatty acid content,

(ii) converting the glycerides into monoesters by transesterification employing one or more monohydric alcohols,

(iii) adsorbing the non-glyceride components onto a selective adsorbent to separate said components from the esters of the oil, and

(iv) thereafter desorbing the non-glyceride components from the adsorbent with the use of solvent to recover said components.

The present method used a selective adsorbent for the adsorption of the minor non-glyceride components from esterified palm oil. We have found that this method is possible because, unlike crude palm oil, esterified plam oil possesses suitable physical and chemical properties. Passage of the esterified palm oil with or without solvent through a selective adsorbent allows solid phase extraction or trapping of carotenoids, sterols, tocopherols and tocotrienols.

It is a surprising discovery that solid adsorbents such as alumina or silica gel, or carbon contrary to the expectation of a skilled worker in the art, provide a very satisfactory way of obtaining the minor non-glyceride components which include the carotenes sterols, tocopherols etc. from the original vegetable oils.

In a typical extraction of carotenes from palm esters (e.g. methyl esters) prepared in accordance with British Patent Specification 2148897A, passage thr ugh bonded phase silica gel provides a recovery of 70% of the available carotenes in the f rm of a

concentrate. Extraction is possible in the presence of alcohols (e.g. methanol, ethanol etc.) from which the esters have been prepared. In a typical extraction of sterols, tocopherols and tocotrienols, palm esters are passed through suitable adsorbents such as activated alumina and silica gel where they are selectively adsorbed and later desorbed using suitable solvents. Purification to pure components can be carried out using conventional chromatographic techniques.

The present method allows for the recovery from palm oil of several valuable minor components the value of which can surpass that of the oil. The industrial preparation of palm esters for oleochemicals, detergents, palm diesel, etc. opens up an important avenue for the recovery of these minor components.

Following is a description by way of example of the recovery of carotonoids, tocopherols, tocotrienols and sterols by reverse phase (C18) silica gel, carbon and alumina adsorbents.

Example 1

Crude palm oil methyl ester was dissolved in methanol (30 ml) and the mixture was introduced into a glass column packed with C_{18} reverse phase (15g) the packing having a height of 20 cm and diameter of 1.8 cm. The ester eluted first and was collected and pumped as fraction W_1. More methanol was introduced into the column to elute out as much ester as possible until carotenoid was about to be eluted out and this was collected and pumped as fraction W_2. Hexane and methanol (98:2 v/v) or chloroform was used to elute out the carotenoid and this was collected as fractrion W_3. Occasionally the column was then cleaned up onc with chloroform (40 ml)

giving rise to fraction W_4. The column was then soaked in methanol for further use. A chromatographic separation was carried out under a nitrogen atmosphere and the recovery of carotenes was determined at 446 nm. The results are shown in Table 1.

Table 1 Recovery of Carotenoids from Methyl Esters of Neutralised Palm Oil using C_{18} Reverse Phase as Adsorbent (I)**

Methyl Esters/g	Solvents used as Eluent*/ml				Collected Fractions/g				Recovery of Carotenoids /%
	A	B	C	D	W_1	W_2	W_3	W_4	
15.19	30	30	15#	40	8.01	4.76	1.76	0.66	66@
15.02	30	45	55#	15	7.98	4.79	1.86	0.0098	90
15.02	30	30	55#	50##	8.49	5.34	1.17	0.0079	63.9
15.01	30	45	45	40	8.42	5.62	0.94	0.0022	95
15.02	30	45	45	40	0.75	11.62	2.36	0.0034	95
8.01	30	190	45	40	1.79	6.08	0.07	0.0597	81

* Consecutive solvents used as eluent were: A = MeOH (use for dissolving ME); B = MeOH; C = n-hexane:MeOH (98:2 v/v); D = $CHCl_3$.
** Weight of adsorbent = 15 g.
# Solvent used was chloroform.
Solvent used was ethanol (95%).
@ This value is based on the 3rd and 4th fractions.

Example 2

The procedure of Example 1 was repeated except that different amounts of starting materials were used, ie. methyl esters had first been eluted and cleaned up by passing through an alumina column. The results are shown in Table 2.

Example 3

The procedure of Example 1 was repeated except that instead of methanol, ethanol was used as eluent and with different amounts of starting materials. The results are shown in Table 3.

Example 4

Recovery of carotenoids, tocopherols, tocotrienols, and sterols from transesterified NPO was carried out by adsorption onto C_{18} reverse phase. SiO_2 followed by alumina.

Neutralised palm oil methyl ester (15g), which was cleaned by passing though Kieselguhr (70-230 Mesh ASTM), was dissolved in methanol (30 ml) and the mixture was introduced into the glass column packed with C_{18} reverse phase (15 g, 20 cm height, 1.8 cm diameter). The eluted ester was collected as fraction 1 (9.20 g). Another 45ml of methanol were introduced into the column to elute out as much ester as possible until carotenoids were about to be eluted out and these were collected as fractions 2 and 3 (3.67 g and 1.91 g respectively). 45 ml of hexane and methanol (98:2 v/v) were used to elute out the

Table 2 Recovery of Carotenoids from Methyl Esters of Neutralised Palm Oil using C_{18} Reverse Phase as Adsorbent (II)**

Methyl Esters@/g	Solvents used as Eluent*/ml				Collected Fractions/g				Recovery of Carotenoids /%
	A	B	C	D	W_1	W_2	W_3	W_4	
15.00	30	30	15#	15	8.30	5.04	1.56	0.0409	74.7
3.76	30	30	45	30	0.17	2.44	1.03	0.1039	80.6
2.10	30	30	45	30	0.05	1.79	0.23	0.0303	39.2
2.01	30	30	45	30	-	1.90	0.13	0.0150	26
1.04	30	30	45	30	0.01	0.91	0.11	0.0144	4

* Consecutive solvents used as eluent were: A = MeOH (use for dissolving ME); B = MeOH; C = n-hexane:MeOH (98:2 v/v); D = $CHCl_3$.
** Weight of adsorbent = 15 g.
# Solvent used was chloroform.
@ These methyl esters samples had first been eluted through an alumina column.

Table 3 Recovery of Carotenoids from Methyl Esters of Neutralised Palm Oil using C_{18} Reverse Phase as Adsorbent (III)**

Methyl Esters/g	Solvents used as Eluent*/ml A	B	C	D	Collected Fractions/g W_1	W_2	W_3	W_4	Recovery of Carotenoids /%
15.02	30	55	55#	-	0.17	14.75	-	-	all@
8.01	30	60	45	30	0.03	7.59	0.12	-	92.3
6.00	30	100	55	40	3.00	2.80	0.10	0.1003	93.6
3.75	30	30	65	30	0.23	2.76	0.67	0.0381	87

* Consecutive solvents used as eluent were: A = 95% EtOH (use for dissolving ME); B = 95% EtOH; C = n-hexane:EtOH (98:2 v/v); D = $CHCl_3$.
** Weight of adsorbent = 15 g.
# Solvent used was chloroform.
@ All carotenoids had been eluted out together with methyl esters in fraction W_2.

carotenoids and this was collected as fraction 4 (0.1785 g). Chromatography separation was carried out under a nitrogen atmosphere. The percentage recoveries of carotenoids (quantified by uv/visible spectrophotometry), tocopherols and tocotrienols (quantified by GLC) of each of the four fractions above are tabulated in Table 4.

Fractions 1, 2 and 3 (total 12.84 g) in which most of the tocopherols, tocotrienols and sterols were found were then combined and eluted into a glass column packed with neutral alumina (1.43 g; ratio of methyl ester:adsorbent, 9:1 w/w). The height of the packing material was 4.5 cm and the diameter of the column was 0.8 cm. The methyl ester eluted was collected as fraction 1 (11.75g). n-Hexane (2 x 12.9ml) was introduced into the column to clean up as much methyl ester as possible and this was collected as fraction 2 (0.90 g). Finally, chloroform (4 x 8.6 ml) was used to recover the sterols, tocopherols and tocotrienols from alumina and this was collected as fraction 3. Chromatography separation was carried out under nitrogen atmosphere. Percentage recoveries of tocopherols and tocotrienols, and sterols in each of the above three fractions were worked out and the results are shown in Table 5.

Example 5

The recovery of carotenoids, tocopherols and tocotrienols, and sterols was performed by adsorption onto alumina followed by C_{18} reverse phase silica gel.

Neutralised palm oil methyl esters (90 g) was cleaned up by filtering through Kieselguhr and then eluted into a glass column packed with alumina (neutral, 10 g, 3.5 cm height and 2.5 cm diameter). The eluted methyl esters were collected as fraction 1

Table 4 R covery of Carotenoids, Tocopherols and Tocotrienols, and Sterols from Methyl Esters of Neutralised Palm Oil using C_{18} Reverse Phase Silica Gel

Fraction	Recovery*/%		
	Carotenoids	Tocopherols and Tocotrienols	Sterols
1	5.2	61.7 (556)	9
2	3.5	19.9 (450)	8.3
3	6.3	11.9 (516)	6.3
4	67.4	2.3 (1045)	ND#

* Recovery in ppm is bracketed.

ND = Not Detectable.

Table 5 Recovery of Tocopherols, Tocotrienols and Sterols from Methyl Esters of Neutralised Palm Oil using Alumina as Adsorbent (I)*

Fraction	Recovery#/%	
	Tocopherols & Tocotrienols	Sterols
1	79.4 (366)	30.1
2	5.8 (347)	18.9
3	2.6 (1510)	15.4

* The methyl esters used has first been eluted through the C_{18} reverse phase column to remove carotenoids as shown in Table 4

Recovery in ppm is bracketed.

(82.23 g). n-Hexane (210 ml) was then introduced into the column to clean up as much methyl esters as possible and this was collected as fraction 2 (6.81 g). Finally, chloroform (240 ml) was used to recover the adsorbed components including tocopherols and tocotrienols, and sterols from the starting material used (i.e. neutralised palm oil methyl esters) in the 3 fractions collected is shown in Table 6.

15.3 g of eluted methyl esters from fraction 1 above was then dissolved in methanol (30 ml) and the mixture was introduced into the glass column packed with C_{18} reverse phase SiO_2 (15 g; 20 cm height, 1.8 cm diameter). The ester eluted was collected as fraction 1 (9.04 g). Another 45 ml of methanol was introduced into the column to elute out as much ester as possible before the carotenoids were eluted out and this was collected as fraction 2 (5.83 g). n-Hexane and methanol (98:2, 45 ml) were used to elute out the carotenoids and collected as fraction 3 (0.32 g). The column was then cleaned up once with chloroform (40 ml) and then soaked in methanol for further use. The chromatography was carried out under nitrogen atmosphere. The percentage recoveries of carotenoids, tocopherols and tocotrienols, and sterols of each of the three fractions above were worked out and tabulated in Table 7.

Table 6 Recovery of Tocoph rols, Tocotrienols and St rols from Methyl Esters of Neutralised Palm Oil using Alumina as Adsorbent (II)

Fraction	Recovery#/%	
	Tocopherols & Tocotrienols	Sterols
1*	77.8 (357)	72.5
2	10.4 (577)	13.2
3	5.7 (3315)	6.8

* A portion of methyl esters eluted was passed through C_{18} reverse phase to recover carotenoids as shown in Table 7.

Recovery in ppm is bracketed.

Table 7 Recovery of Car tenoids, T copherols and Toc trienols, and St rols fr m Methyl Esters of Neutralised Palm Oil using C_{18} Reverse Phase as Adsorbent*

Fraction	Recovery#/%		
	Carotenoids	Tocopherols and Tocotrienols	Sterols
1	6.04	71.6 (423)	42.5
2	9.72	33.7 (316)	12.3
3	89.06	1.7 (290)	ND

* The methyl esters used has first been eluted through the alumina column as shown in Table 6.

The percentage recovery was based on the starting materia used in the column. Recovery in ppm is bracketed.

ND = Not Detectable.

It is understood that in place of the methyl alcohol used to produce the above described methyl esters any of the branched or straight chain alcohol having from 1 to 6 carbon atoms may be used, although methyl alcohol is preferred.

Table 8 Adsorption and Extraction of Carotenoids of Methyl Esters of Crude Palm Oil using Activated Carbon - Continuous Column Extraction*

Experiment	Adsorption of Carotenoids/%	Recovery of Carotenoids/%
1#	79	49
2##	88	50.6

* The following conditions were used:- weight of methyl esters = 5 g; weight of carbon = 1 g; ratio of methyl esters to carbon = 5:1; weight of butylated hydroxytoluene (BHT) = 0.01 g; adsorption was done at 28-30°C; percentage recovery of carotenoids from carbon was from toluene fraction only.

18 ml of petroleum ether b.p. 60-80°C, 88 ml of toluene and 38 ml of toluene/ethanol (3:1 v/v) successively were used as eluent; all solvents were at 28-30°C.

10 ml of n-hexane (of which 5 ml was used to dissolve methyl esters), 43 ml of toluene and 17 ml of toluene/ethanol were used as eluent; both toluene and toluene/ethanol were pre-warmed to 40°C before use.

Table 9 Adsorption and Extraction of Carotenoids of Methyl Esters of Crude Palm Oil using Activated Carbon - Batchwise Extraction*

Exp.	Activated Carbon	Solvent for Recovery/ml	Adsorption of Carotenoids/%	Recovery of Carotenoids/%	Remarks
1	Carbon S511	CH_2Cl_2; 120	25.2	3.8	Untreated carbon; Carbon pH = 11.8
2	Norit OL	CH_2Cl_2; 120	61.2	3.3	Untreated carbon
3	Carbon S511	CH_2Cl_2; 120	70.3	3	Carbon was warmed and vacuum pumped dry before use
4	Carbon S511	CH_2Cl_2; 120	73.5	2.4	Antioxidant hydro quinone; carbon was vacuum pumped dry before use.
5	Carbon S511	CH_2Cl_2; 120	66.5	10.3	Carbon - treated with EtOH, HCl & Na_2CO_3; activated at 300°C; pumped dry at 200°C; pH = 10.1
6//	Carbon S511	Toluene; 60	61.9	26.1	Carbon was pumped at 250°C for 2 hr before use.

* The following conditions were used: weight of methyl esters = 20 g; weight of carbon = 4 g; methyl esters:carbon = 5:1; adsorption of material onto carbon was done at 28-30°C; recovery of carotenoids from carbon was carried out using a Soxhlet extractor with solvent.

// Recovery of carotenoids from carbon was done by soaking the carbon in toluene.

CLAIMS

1. A method for the isolation of the minor non-glyceride components of palm oil or like vegetable oil containing free fatty acid and non-glyceride components similar to that of palm oil, which method comprises:

(i) esterifying the free fatty acid component of the oil with one or more monohydric alcohols to form an esterified oil with a very low free fatty acid content,

(ii) converting the glycerides into monoesters by transesterification employing one or more monohydric alcohols,

(iii) adsorbing the non-glyceride components onto a selective adsorbent to separate said components from the esters of the oil, and

(iv) thereafter desorbing the non-glyceride components from the adsorbent with the use of solvent to recover said components.

2. A method as claimed in claim 1 wherein the adsorbent is activated alumina carbon or silica gel, preferably reverse phase (particularly C 18) silica gel.

3. A method as claimed in claim 1 or claim 2 wherein the non-glyceride components obtained from step (iii) are separated into sterols, tocopherols, tocotrienols and carotenes by a chromatographic technique, or wherein the recovered minor component is only carotene by using carbon adsorbent.

4. A method as claimed in any one of the preceding claims wherein the esterification of step (i) is carried out employing (a) a solid alkali

metal bisulphate r (b) a sulphate acid strongly-acidic ion-exchange resin as a catalyst and the transesterification of step (ii) is carried out employing a basic catalyst or both the esterification and transesterification are carried out using an enzyme e.g. candida rugosa.

5. A method as claimed in any one of the preceding claims wherein the oil which is esterified in step (i) is a palm oil or a palm oil fraction.

6. A method as claimed in any one of the preceding claims wherein the carboxylic acid is esterified and/or the glycerides are transesterified with one or more C_1 to C_3 alcohols, preferably methanol.

7. A method as claimed in any one of claims 4 to 6 wherein there is employed from 1 to 20% by weight of catalyst, based upon the weight of the free fatty carboxylic acid.

8. A method for the recovery of carotenes from esterified palm oil by employing a C 18 reverse phase silica gel as adsorbent using two combinations of solvents as consecutive eluents as follows :

(i) Methanol, n-hexane : methanol (98 : 2 v/v) and $CHCl_3$

(ii) Ethanol, n-hexane : 95% EtOH (98 : 2 v/v) and $CHCl_3$.

9. A method as claimed in claim 9 in which the recovery of carotenes from esterified palm oil is at 1 ast 95% with a ratio of methyl ester to adsorbent of 1 : 1 (w/w) on the r covery of carotenes from esterfied palm il is at least 92.3% when the

ratio of methyl esters to adsorbent is o.5 : 1 (w/w).

10. A method for the recovery of carotenes from esterified plam oil by employing activated carbon as adsorbent and using aromatic solvent such as toluene or aliphatic solvent such as dichloromethane or ethanol for desorbing carotenes from the carbon.

11. A method for the isolation of the minor non-glyceride components of palm oil or the like substantially as hereinbefore described in any one of the examples.

12. A non-glyceride component of palm oil or the like when obtained using a process as claimed in any one of claims 1 to 11.

13. Sterols, tocopherols, tocotrienols and carotenes when obtained from the component of claim 12.

Published 1989 at The Patent Office, State House, 66/71 High Holborn, London WC1R 4TP. Further copies may be obtained from The Patent Office. Sales Branch, St Mary Cray, Orpington, Kent BR5 3RD. Printed by Multiplex techniques ltd, St Mary Cray, Kent, Con. 1/87